

Claims:

1. A recombinant hG-CSF-L-vFc fusion protein comprising hG-CSF, a peptide linker, and a human IgG Fc variant, wherein the human IgG Fc variant comprises a hinge, CH2, and CH3 domains of human IgG4 with Ser228Pro and Leu235Ala mutations mutation as SEQ ID NO 20.
2. The recombinant hG-CSF-L-vFc fusion protein of claim 1, wherein the peptide linker (i) comprises about 20 or fewer amino acids; (ii) is present between hG-CSF and the human IgG Fc variant; and (iii) comprises two or more amino acids selected from the group consisting of glycine, serine, alanine, and threonine.
3. The recombinant hG-CSF-L-vFc fusion protein of claim 1, wherein the hG-CSF-L-vFc fusion protein is characterized by an enhanced *in vitro* biological activity of at least 2 fold relative to that of rhG-CSF on a molar basis.
4. A CHO-derived cell line producing the hG-CSF-L-vFc fusion protein of claim 1 in the cell line's growth medium in excess of 10 µg per million cells in a 24 hour period.
5. The CHO-derived cell line producing the hG-CSF-L-vFc fusion protein of claim 4 in the cell line's growth medium in excess of 30 µg per million cells in a 24 hour period.
6. A method for making a recombinant fusion protein comprising hG-CSF, a flexible peptide linker, and a human IgG Fc variant, which method comprises: (a) generating a CHO-derived cell line by transforming the CHO cell line with a gene encoding the recombinant fusion protein comprising hG-CSF; (b) growing the cell line under conditions sufficient for expressing the recombinant fusion protein in its the cell line's growth medium at a rate of in excess of 10 µg per million cells in a 24 hour period; and (c) purifying the expressed protein from step (b), wherein the recombinant fusion protein is characterized by an enhanced *in vitro* biological activity of at least 2 fold relative to that of rhG-CSF on a molar

basis; and wherein the human IgG Fc variant comprises a hinge, CH2, and CH3 domains of human IgG4 with Ser228Pro and Leu235Ala mutations mutation as SEQ ID NO 20.

- 5 7. The method of claim 6, wherein in step (b) growing the cell line under conditions sufficient for expressing the recombinant fusion protein in the cell line's growth medium at a rate of in excess of 30 µg per million cells in a 24 hour period.
- 10 8. The method of claim 6, wherein the flexible peptide linker (i) comprises about 20 or fewer amino acids; (ii) is present between hG-CSF and the human IgG Fc variant; and (iii) comprises two or more amino acids selected from the group consisting of glycine, serine, alanine, and threonine.
- 15 9. The method of claim 8, wherein in step (b) growing the cell line under conditions sufficient for expressing the recombinant fusion protein in the cell line's growth medium at a rate of in excess of 30 µg per million cells in a 24 hour period.
- 20 10. A method for making a recombinant fusion protein comprising hG-CSF, a flexible peptide linker, and a human IgG Fc variant, which method comprises: (a) generating a CHO-derived cell line by transforming the CHO cell line with a gene encoding the recombinant fusion protein comprising hG-CSF; (b) growing the cell line under conditions sufficient for expressing the recombinant protein in the cell line's growth medium at rate of in excess of 10 µg per million cells in a 24 hour period; and (c) purifying the expressed protein from step (b), wherein the recombinant fusion protein is characterized by an enhanced *in vitro* biological activity of at least 2 fold relative to that of rhG-CSF on a molar basis; wherein the flexible peptide linker (i) comprises about 20 or fewer amino acids; (ii) is present between hG-CSF and the human IgG Fc variant; and (iii) comprises two or more amino acids selected from the group consisting of glycine, serine, alanine, and threonine; and wherein the human IgG Fc variant comprises a hinge, CH2, and CH3 domains selected from the group consisting of human IgG4 with Ser228Pro and Leu235Ala mutations as SEQ ID NO 20.
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11. The method of claim 10, wherein in step (b) growing the cell line under conditions sufficient for expressing the recombinant fusion protein in the cell line's growth medium at a rate of in excess of 30 μ g per million cells in a 24 hour period.